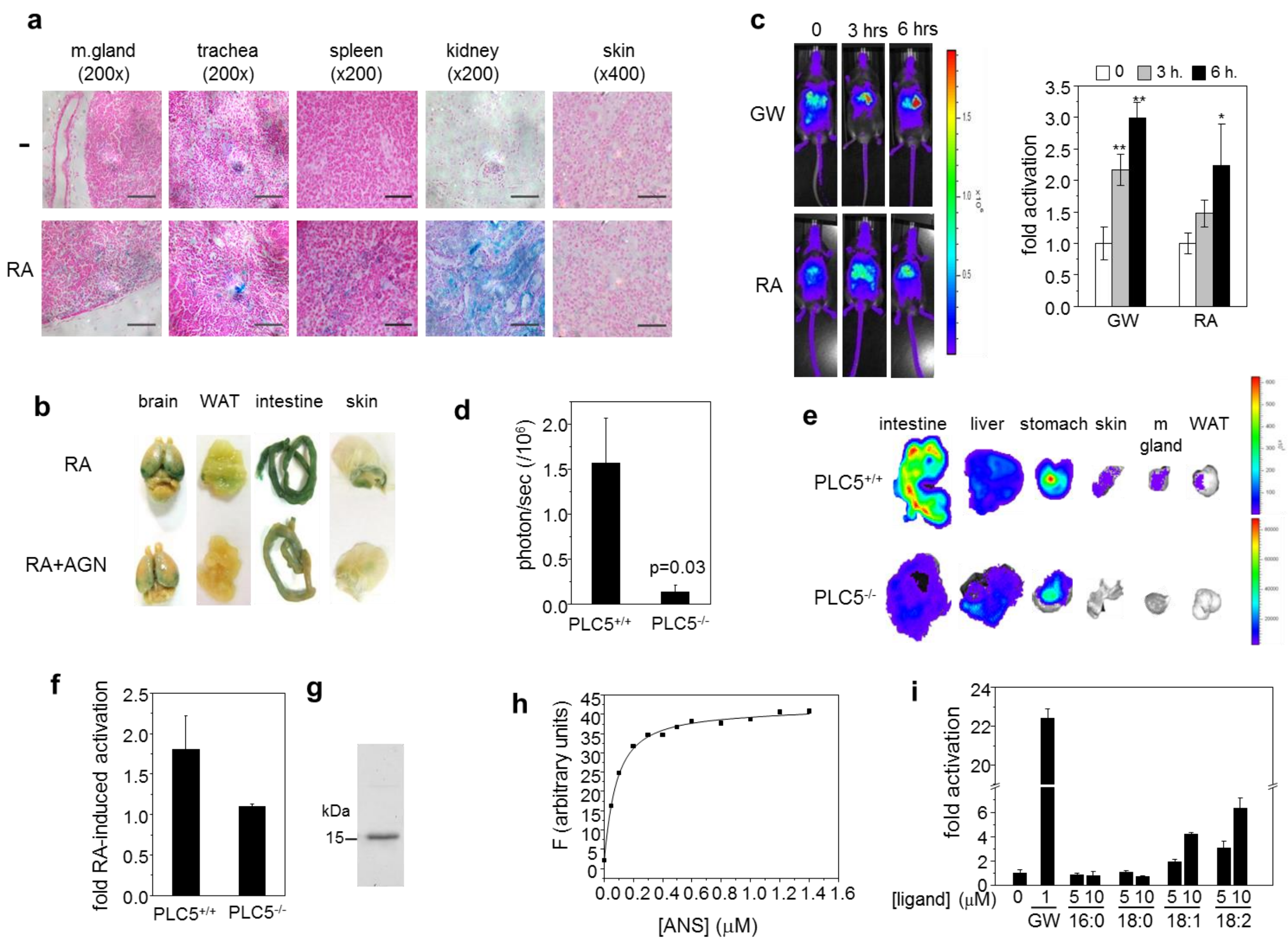
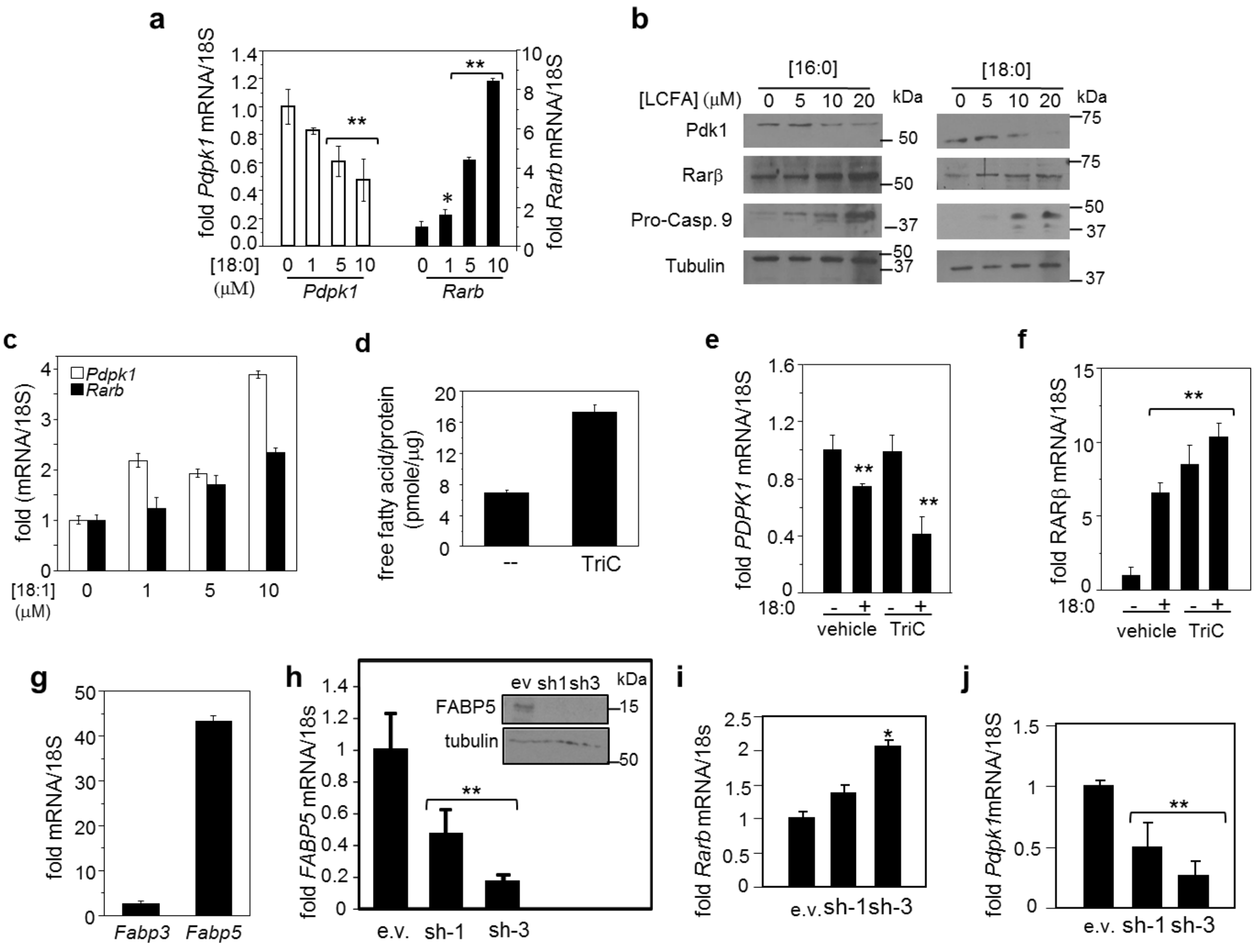
Supplementary Information



Supplementary Figure 1: RA activates PPARβ/δ *in vivo*

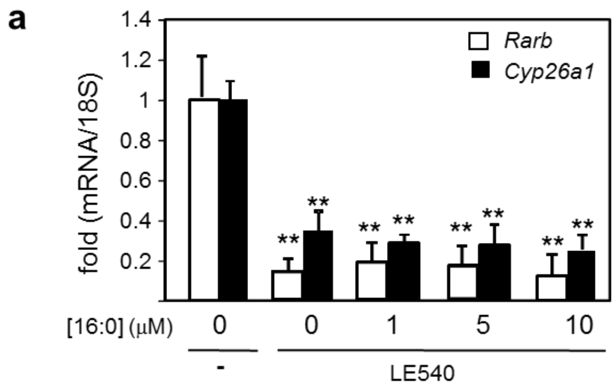
a) X-gal staining of tissue sections harvested from RARE-LacZ mice treated with vehicle (-) or RA (1 mg). Representative images out of 6 mice per group are shown. Scale bars represent 50 µm in skin sections and 100 µm in all other tissue sections. **b**) X-gal staining of tissues harvested from RARE-LacZ mice treated with RA (1 mg) or co-treated with RA and the pan-RAR antagonist AGN193109 (AGN; 1mg). Representative images out of 3 mice per group are shown.

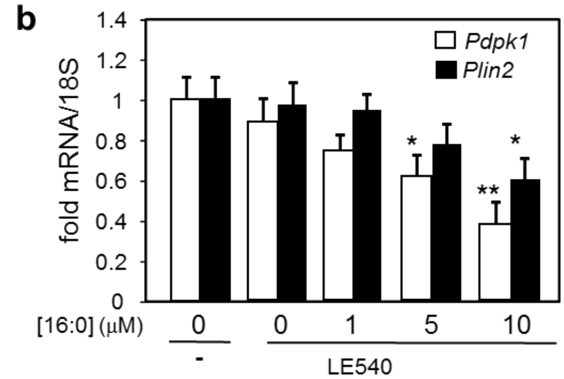
c) PPRE-luc mice were injected with vehicle, the PPARβ/δ agonist GW1516 (GW), or RA. Left: representative images, out of 3 mice, of luciferase activity in mice 3 and 6 h. following injection using IVIS 200 CCD camera. Right: images were quantified using the software Living Image (Xenogen; right). Data are mean±SEM (n=3). *p<0.05, **p<0.01, calculated by unpaired t-test. **d**) Quantification of whole body imaging of *PPRE-luc*$^{+/-}$/*FABP5*$^{-/-}$ (*PLC5*$^{+/+}$, n=3) and *PPRE-luc*$^{+/-}$/*FABP5*$^{+/+}$ (*PLC5*$^{-/-}$, n=4) mice. Data are mean±SEM. P-value was calculated by unpaired t-test. **e**) Representative images of organs harvested from *PPRE-luc*$^{+/-}$/*FABP5*$^{-/-}$ (*PLC5*$^{-/-}$, *n=4*) and *PPRE-luc*$^{+/-}$/*FABP5*$^{+/+}$ (*PLC5*$^{+/+}$, *n=3*) mice. **f**) Fold enhancement of whole body luminescence of *PLC5*$^{+/+}$ (n=3) and *PLC5*$^{-/-}$ mice (n=4) mice following treatment with RA. Data are mean±SEM. **g**) Recombinant his-tagged mFABP5 expressed in *E. coli* and purified. **h**) Representative fluorescence titration out of 3, demonstrating binding of ANS to FABP5. **i**) Transactivation assays were carried out using COS-7 cells co-transfected with vectors encoding PPARβ/δ, a luciferase reporter driven by a PPAR response element (PPRE), and a vector harboring β-galactosidase, serving as a transfection control. Cells were treated with the denoted ligands for 18 h., and luciferase activity was measured and corrected for β-galactosidase activity. Data are mean±SD (n=3).



Supplementary Figure 2: Differential effects for SLCFA and ULCFA on transcriptional activities of PPARβ/δ and RAR

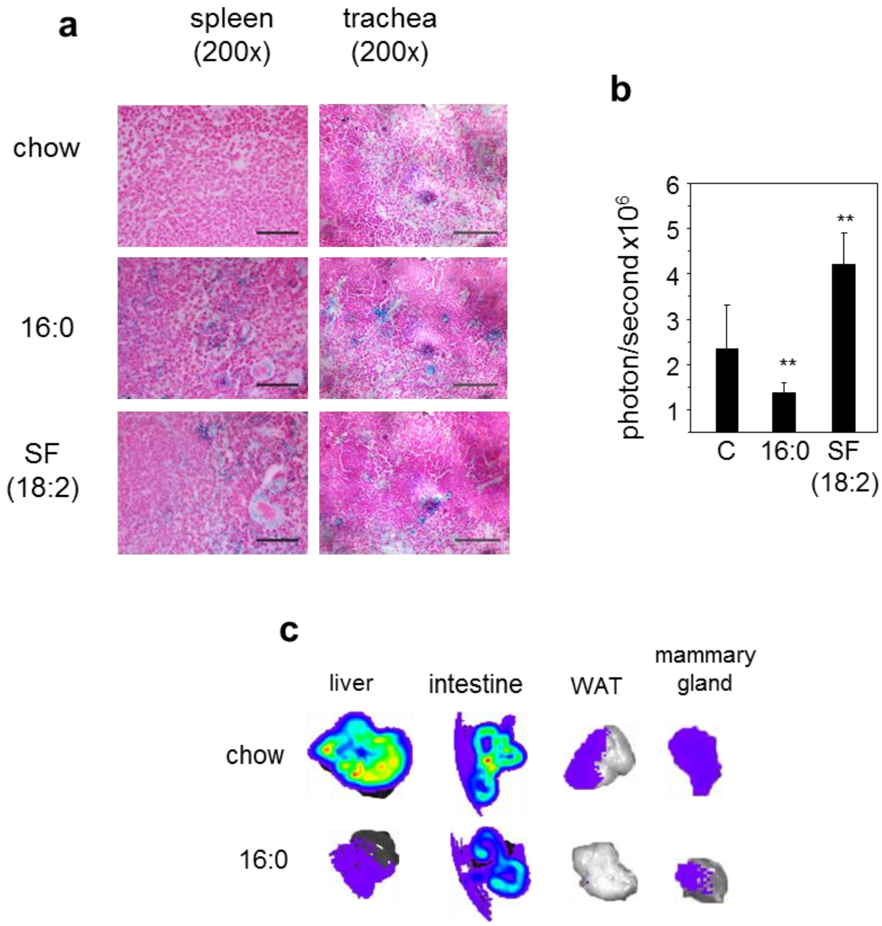
a) Levels of mRNA for PPARβ/δ target gene, *Pdpk1*, and RAR target gene *Rarb* in NaF cells treated with 18:0 for 6 h. Data are mean±SD (n=3). *p<0.05, **p<0.01, calculated by unpaired t-test. **b**) Representative immunoblots demonstrating levels of PPARβ/δ target Pdk1, and RAR targets Rarβ and caspase 9 in NaF cells treated with 16:0 or 18:0 for 18 h. (n=3). **c**) Levels of *Rarb* and *Pdpk1* mRNA in NaF cells treated with 18:1 for 6 h. Data are mean±SD (n=3). **d**) Concentrations of free FA (FFA) in NaF cells treated with vehicle (-) or with Triacsin C (TriC, 5 μM) for 6 h. Data are mean±SD (n=3). **e**), **f**) Levels of mRNA for *Pdpk1* (d), or *Rarb* (e) in NaF cells treated with vehicle or 18:0 (10 μM) in the absence or presence of TriC (5 μM) for 6 h. Data are mean±SD (n=3). **p<0.01, calculated by unpaired t-test. **g**) Relative levels of *Fabp3* and *Fabp5* mRNAs in NaF cells. Data are mean±SD (n=3). **h**) Level of *Fabp5* mRNA in two NaF cell lines stably expressing FABP5 shRNA (sh-1 and sh-3) compared to parental cells expressing an empty vector (e.v.). Data are mean±SD (n=3). **p<0.01, calculated by unpaired t-test. Inset: representative immunoblots demonstrating reduced level of FABP5 in NaF lines expressing FABP5shRNA. (n=3). **i**), **j**) Levels of *Rarb* (i) and *Pdpk1* (j) mRNAs in NaF cells expressing varying levels of FABP5**.** Data are mean±SD (n=3). *p<0.05, **p<0.01, calculated by unpaired t-test.





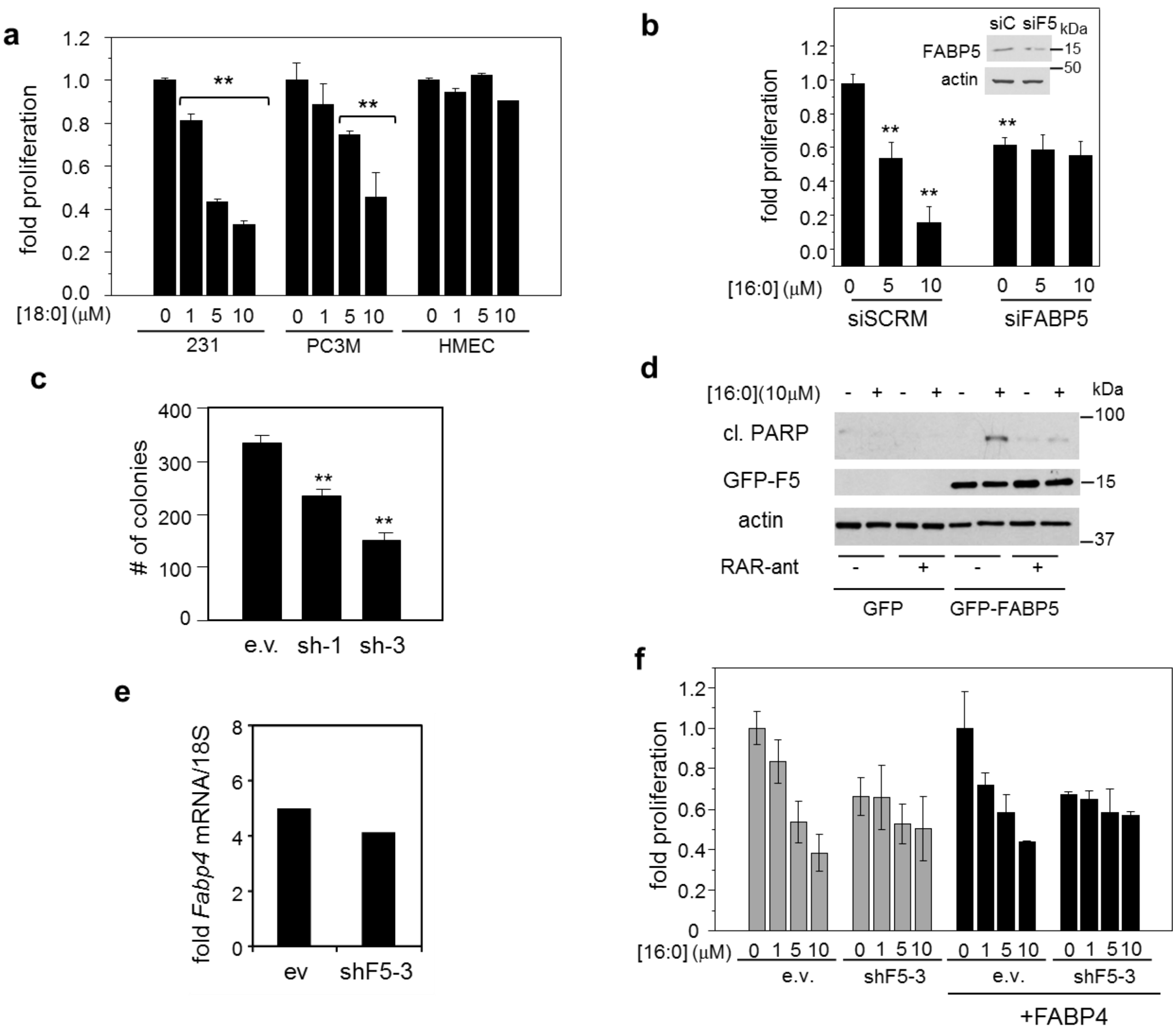
Supplementary Figure 3: LCSFAs regulates the transcriptional activity of RAR and PPARβ/δ in an RA-dependent manner

a), **b**) Levels of RAR target genes *Rarb* and *Cyp26a1* mRNA (a), or PPARβ/δ target genes *Pdpk1* and *Plin2* (b) in NaF cells treated with 16:0 in the presence of the pan-RAR antagonist LE540 (1 μM) for 6 h, vs. an untreated control. Data are mean±SD (n=3). *p<0.05, **p<0.01, calculated by unpaired t-test.



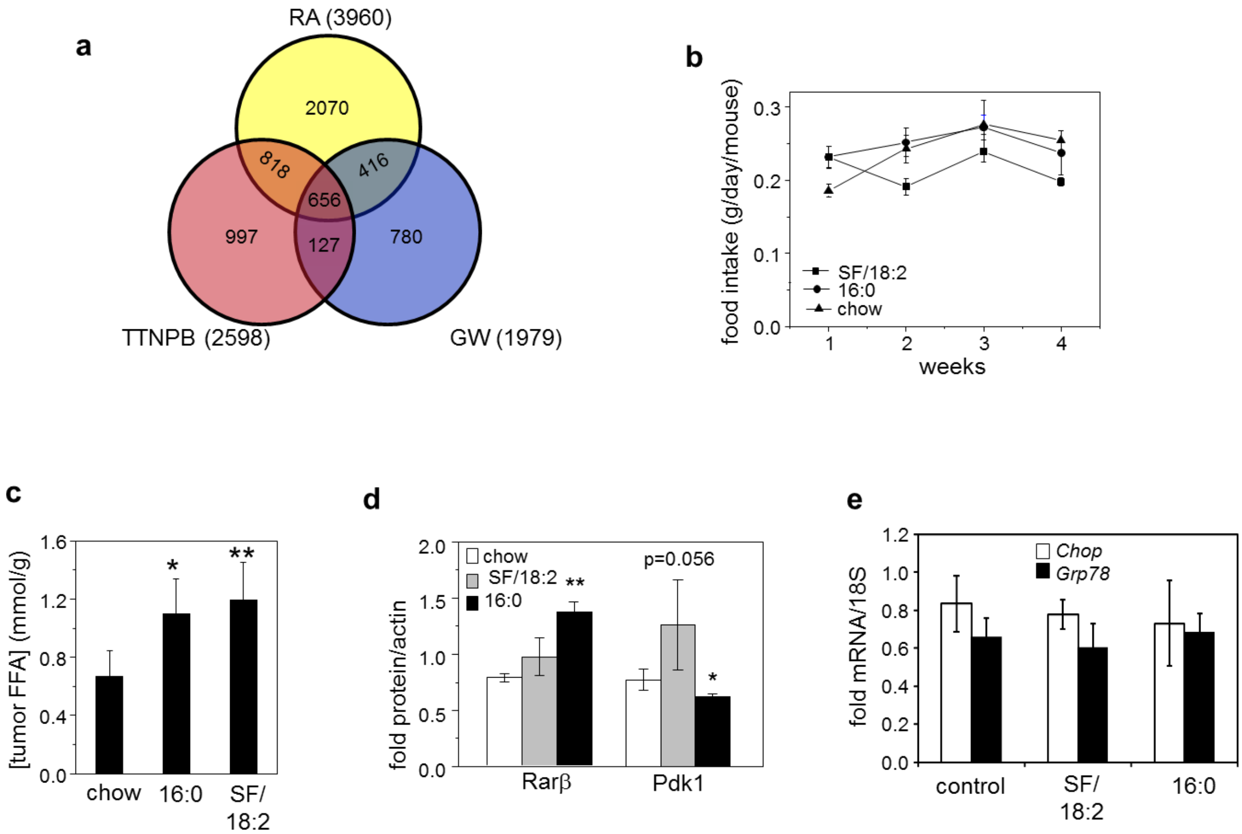
Supplementary Figure 4: Dietary LCFAs activate RAR and PPARβ/δ *in vivo*

a) Representative images of x-gal staining in sections of tissues harvested from RARE-LacZ mice fed denoted diets for a week (n=6). Scale bars represent 100 µm. **b**) Quantification of whole body imaging of PPRE-luc mice fed the different diets for a week. Data are mean±SEM (n=6). **p<0.01, calculated by unpaired t-test. **c**) Representative images of organs harvested from PPRE-luc mice fed 16:0-enriched diet or regular chow (n=6 for each group) for a week prior to treatment with RA.



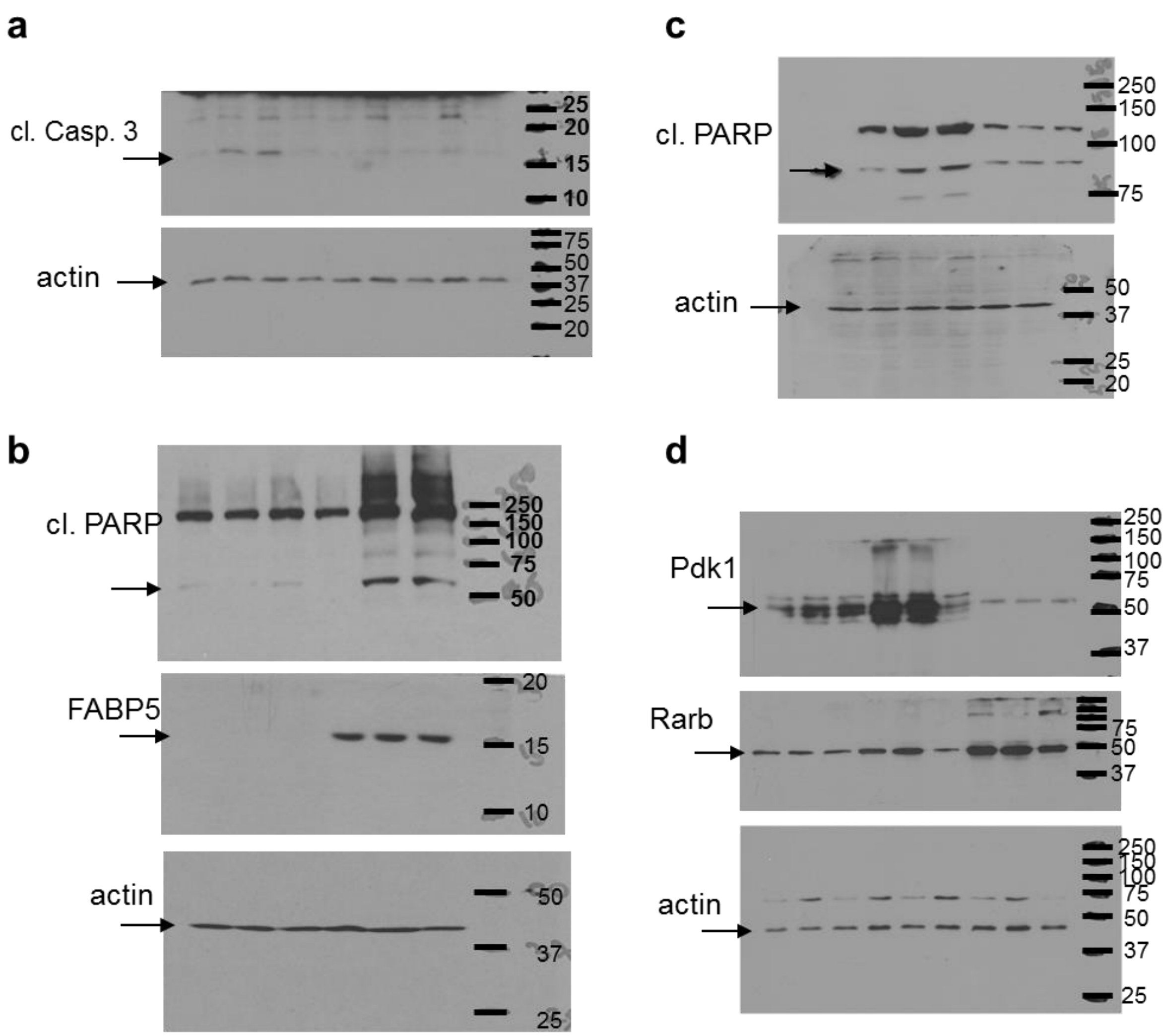
Supplementary Figure 5: FABP4 does not compensate for reduced expression of FABP5

a) Effect of 18:0 on proliferation of MDA-MB-231, PC3M and HMEC cells. Cells were treated with denoted concentrations of 18:0 for 4 days and proliferation assessed using MTT assays. Data are mean±SD (n=4). **p<0.01, calculated by unpaired t-test. **b**) Proliferation assay in NaF cells transiently transfected with FABP5 siRNA of non-targeting siRNA (siSCRM) and treated with 16:0 for 4 days. Data are mean±SD (n=3). **p<0.01 vs. untreated siRNA control, calculated by unpaired t-test. Insert: representative immunoblots demonstrating reduced level of FABP5 in cells expressing FABP5 siRNA (siF5) compare to the control siRNA (siC) (n=3). **c**) Number of colonies formed in soft agar by NaF lines with varying expression levels of FABP5 (Fig. 4c). Data are mean±SD (n=3). **p<0.01 vs. e.v. expressing cells, calculated by unpaired t-test. **d**) Immunoblots of cleaved PARP in MCF-7 cells overexpressing GFP-FABP5 or GFP control and treated with 16:0 for 4 days in the presence or absence of antagonists for RARα (BMS19614), RARβ (LE135), or RARγ (MM11253) (1 μM each). Immunoblots are representative of 2 independent experiments. **e**) Levels of *Fabp4* mRNA in NaF lines stably expressing e.v. of FABP5shRNA (Fig S2i) transfected with a plasmid harboring c-myc-tagged FABP4. Representative data out of 2 independent experiments. **f)** Effect of 16:0 on proliferation of NaF lines stably expressing e. v. or a vector harboring FABP5 or ectopically expressing e.v. or a vector encoding FABP4. Cells were treated with 16:0 for 4 days and proliferation assessed by MTT assays. Data are mean±SD (n=3).



Supplementary Figure 6: 16:0 suppresses mammary tumor growth *in vivo* by shifting RA signalling and not by inducing ER stress

a) Venn diagram illustrating the total number of genes regulated by RA, TTNPB or GW, and the overlap between them. **b**) Food intake of mice fed control chow (chow), a diet enriched with 16:0, or a diet enriched in safflower oil (SF/18:2). Data are mean±SEM (n=6). **c**) Concentrations of free FA in tumors of mice fed denoted diets. Data are mean±SD (n=6). *p<0.05, **p<0.01, calculated by unpaired t-test. **d**) Quantification of immunoblot showing levels of Pdk1 and Rarβ in tumors that arose in mice fed the different diets. Data are mean±SD (n=3). *p<0.05, **p<0.01, calculated by unpaired t-test. **e**) Expression levels of the ER stress markers *Chop* and *Grp78* in tumors that arose in mice fed the denoted diets. Data are mean±SD (n=5).



Supplementary Figure 7: Uncropped blots
a) Uncropped blots from Figure 4g.
b) Uncropped blots from Figure 4j.
c) Uncropped blots from Figure 4m.
d) Uncropped blots from Figure 6e.